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Fractionation and characterization of gum from *Acacia tortuosa*. Effect of enzymatic and alkaline treatments

Olga Beltrán[a,*], Gladys León de Pinto[a], Maritza Martínez[a], Luc Picton[b], Céline Cozic[b], Didier Le Cerf[b], Guy Muller[b]

[a]*Centro de Investigaciones en Química de los Productos Naturales, Facultad de Humanidades y Educación, Universidad del Zulia, Apartado 526, Maracaibo, Venezuela*
[b]*Laboratoire 'Polymères, Biopolymères et Membranes', UMR 6522 CNRS Université de Rouen, 76 821 Mont Saint Aignan Cedex, France*

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Abstract

The polysaccharide from *Acacia tortuosa* (Gummiferae ssp) has been characterized using size exclusion chromatography (SEC) with multiangle laser light scattering (MALLS). Comparison of the elution profile of the native gum with those exhibited by the gum after basic and enzymatic hydrolyses showed interesting modifications. Four distinct populations have been observed. The polysaccharide consists of a mixture of arabinogalactan (AG) and a complex arabinogalactan–protein (AGP)as has been reported for *Acacia Senegal* gum (Arabic gum).
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Keywords: Acacia tortuosa; Gum exudate; Molecular weight distribution; Arabinogalactan–protein

1. Introduction

Acacia tortuosa, a tropical American Gummiferae species, produces a clear gum highly soluble in water. Analytical data and the relevant structural features of the polysaccharide isolated from Venezuelan *Acacia* gum have been reported (León de Pinto, Martínez, Ortega, Villavicencio, & Borjas, 1993; León de Pinto, Martínez, Galindo de Bolaño, & Igartuburu, 1997). Many analytical data were basically similar to other Gummiferae *Acacia* gums but differed in the sugar composition. It was demonstrated that the presence of traces of xylose and rhamnose was not observed. A combination of chemical methods with ^{13}C NMR spectroscopy supported that the core of the structure is mainly a branched β (1→3) galactan. Arabinose and the uronic acid residues could not be totally removed from the core. Arabinose (as furanose and pyranose residues) exists as terminal and 3-*O*-linked, while xylose is present as terminal residues.

Characterization of water-soluble polymers mixture has become important in a number of scientific and technological disciplines. Traditionally, size exclusion chromatography (SEC) has been applied to separate various mixtures of proteins, nucleic acids and polysaccharides (Chmelík, Chmelíková, & Novotny, 1997). Nevertheless, proper analysis of SEC requires using adequate polymer standards with chemical structure similar to that of the analyte. This condition is no more necessary by using SEC coupled on line with a multiangle laser light scattering (MALLS) detection.

This work deals with the molecular characterization of *Acacia tortuosa* gum using SEC-MALLS analysis. We have particularly focused the attention on specific enzymatic and alkaline treatments of such gum.

2. Materials and methods

2.1. Origin and purification of gum sample

Gum from *Acacia tortuosa* wild, known in Venezuela as uveda, was collected in the location of 'Los Puertos de Altagracia', East of Maracaibo lake, Zulia state, Venezuela,

* Corresponding author. Tel.: +58 261 7596269; fax: +58 261 7596204.
E-mail address: obeltran@luz.edu.ve (O. Beltrán).

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by the authors in no rainy season (February–April 2002). Taxonomic identification was done by Dr Lourdes Cardenas, Botanical taxonomist. The gum exudates very soluble in water, were purified by dissolution, dialysis and freeze-dried.

2.2. Alkaline treatment

The original gum (5 g) was hydrolyzed with a saturated barium hydroxide solution (200 ml) at 100 °C for 8 h. The hydrolyzed gum was neutralized with sulfuric acid (1 M), filtrated and freeze-dried.

2.3. Enzymatic treatment

The gum sample (250 mg) was dissolved in deionized water (25 ml) and the pH was adjusted (7.5) with NaOH (0.1 M). It was added pronase (1 ml, 3.4%) to the gum solution (9 ml) and incubated overnight at 37 °C.

2.4. Analytical techniques

The absolute determination of molecular weight and size distributions (MWD and RHD, respectively) were performed by coupling on line a SEC to a multi-angle laser light scattering (MALLS) and a differential refractive index detector (DRI). The light scattering signal is proportional to the product of concentration and molecular weight whereas the DRI signal is proportional only to the concentration.

2.5. Size exclusion chromatography

The solution of 0.1 M $LiNO_3$, used as carrier, was filtered through 0.1 μm filter (Millipore) degassed (ERC 413) previously, eluted at 0.5 ml min^{-1} flow rate (Flom HPLC pump 301) and clarified through a 0.45 μm filter unit upstream columns. The sample was injected through a 100 μl full loop. The size exclusion chromatography (SEC) line consisted of an OHPAK SB-G guard column as protection and two OHPAK SB 804 and 806 HQ columns (Shodex) in series. The column packing is a polyhydroxymethylmethacrylate gel.

2.6. Multi angle laser light scattering (MALLS)

The MALLS photometer, a DAWN—Enable Optical System (EOS) from Wyatt technology Inc. (Santa Barbara, USA) is fitted with a K5 cell with 18 photodiodes and He–Ne laser (λ = 690 nm). The QELS detector from Wyatt technology is connected to 115° angle of the MALLS detector. The collected data were analyzed using the Astra V-4.85 software package. The SEC-MALLS technique has been described previously (Picton, Bataille, & Muller, 2000). The concentrations of each eluted fraction have been determined with the DRI (ERC 7515A) according to a classical value used for polysaccharides of dn/dc (0.15 ml g^{-1}). The samples were dissolved (about 5 g l^{-1}) in the 0.1 μm filtered carrier ($LiNO_3$ 0.1 M + NaN_3 0.02%, water from Milli-Q water reagent system) and gently stirred during 5 h, then filtered through 0.45 μm type membrane (Millipore).

Molar masses (from static light scattering) and hydrodynamic radii (R_h from quasi elastic light scattering) have been reported in the following results. The use of QELS apparatus is fully justified by the fact that quite the whole studied samples presents gyration radius (R_g) not large enough to be measured. Effectively, the low dimensions of tortuosa gum species ($R_g < \lambda/20$, where λ is the wavelength of the laser) lead to light scattering angular dependences which are too small to measure the slope thus the R_g.

3. Results and discussion

The elution profile of *Acacia tortuosa* gum is shown in Fig. 1 wherein are represented the distribution of both molar masses (MWD) and hydrodynamic radii (RHD) as a function of elution volume together with light scattering (The signal from 90° LS photodiode detector) and DRI response. Four distinct populations are detected. The first population (13.0–15.4 ml) is representative of low concentrated and high molar masses species as indicated by DRI and LS responses.

The second (15.4–17.7 ml) and third (17.7–19.0 ml) populations have high DRI but low LS responses. These features indicate high concentration of lower masses species than those present in the first population. The fourth population (19.0–21.0 ml) shows low DRI and low LS responses as indication of both low concentration and molar masses.

Physicochemical characteristics of *Acacia tortuosa* native gum, obtained by the application of SEC/MALLS are shown in Table 1. It is important to know that the given results in terms of molar masses and proportions have to be considered with attention and caution as the separation was not very efficient. Nevertheless, the whole gum results indicated values of 410,000 and 170,000 g mol^{-1} for weight average molar mass ($\overline{M_w}$) and number average molar mass ($\overline{M_n}$), respectively. The high polydispersity index (2.4) of the native gum suggests a very large spectrum of molar masses species (Fig. 1), which is probably consistent with a very complex system. This observation is fully confirmed by the detailed average molar masses of each population given in Table 1.

Studies of the well-known *Acacia senegal* (arabic gum) and *Acacia seyal* gum have attributed high molar masses fraction to an arabinogalactan-protein (AGP) complex (Picton et al., 2000; Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005). A way to evidence AGP consists in a pronase treatment leading to a destruction of such species (Connolly, Fenyo, & Vandevelde, 1987). Elution profiles

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